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HISTAMINE RELEASE FROM HUMAN RIGHT ATRIUM

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Histamine release has been detected biologically after exposure of human right atrial biopsy samples to compound 48/80. Human right atrial samples contain large quantities of histamine, 1035 ± 65 ng/g fresh weight. Exposure of untreated electrically paced atrial strips to compound 48/80, 300 μg/ml, caused an increase in the force of atrial contraction similar to that caused by histamine. Pretreatment of strips with cimetidine, 3.16×10^{-5} M, prevented the inotropic response to compound 48/80. It is concluded that mast cell degranulation in human atrial tissue can cause sufficient release of histamine to modify the function of the heart.

Introduction Histamine, which is stored in mast cells within the heart (Giotti, Guidotti, Mannaioni & Zilletti, 1966), produces large changes in cardiac function in most animal species (Levi, Allan & Zavecz, 1976; Owen, 1977; Flynn, Gristwood & Owen, 1979) including man (Gristwood, Lincoln & Owen, 1980a; Boyce, Owen & Wareham, 1980). Studies in guinea-pigs have demonstrated that procedures which cause mast cell degranulation e.g. anaphylaxis (Levi, 1972; Capurro & Levi, 1975; Gristwood, Owen & Smith, 1980b) and compound 48/80 (Pöch & Kukovetz, 1967) release histamine from the heart and simultaneously cause large changes in cardiac function. The important contribution of histamine to the cardiac response following mast cell degranulation is apparent from the reduction or abolition, by histamine H_2-receptor antagonists, of most aspects of cardiac anaphylaxis (Capurro & Levi, 1973; Levi *et al.*, 1976; Gristwood *et al.*, 1980b).

In the present study, we have measured the histamine content of human right atrial samples and determined whether histamine can be released from the human heart in quantities adequate to change cardiac function.

Methods Human right atrial biopsy samples were obtained during cardiac surgery for organ bath studies as described previously (Gristwood *et al.*, 1980a). For determination of histamine contents, samples upon removal were placed immediately in polythene containers and frozen in liquid nitrogen; these were then kept at below −30°C before assay.

Histamine content of human right atrium The histamine content of biopsy samples of right atrium was assayed by the double isotope radioenzymatic method (as reviewed by Beaven & Horakova, 1978) in which histamine is converted into radiolabelled N^{τ}-methylhistamine, the methylation being catalysed by the enzyme, histamine *N*-methyltransferase. S-adenosyl-L-[methyl-^{3}H]-methionine (0.5 μCi, 500 mCi/mmol; Radiochemical Centre, Amersham, Bucks) was used as methyl group donor. A trace amount of [^{14}C]-histamine (1200 d/min, 0.2 ng, Radiochemical Centre, Amersham, Bucks) was included in the incubation mixture to monitor efficiency of enzymatic methylation and of extraction of the product, N^{τ}-methylhistamine. The incubation and extraction procedure was essentially that previously described (Beaven & Horakova, 1978) except that, after the extraction procedure, the chloroform extracts were taken to dryness in a vacuum desiccator. The tissue (ca. 100 mg) was homogenized in 2 ml of phosphate buffer (0.1 M, pH 7.9) at 0–4°C. The supernatant obtained by centrifuging the homogenate at 10000 *g* for 1 min was assayed directly, or where necessary, after dilution with phosphate buffer. We checked that histamine was not metabolized during homogenization using guinea-pig heart tissue which was homogenized as described above in phosphate buffer which contained [^{14}C]-histamine (1.11×10^6 d/min, 185 ng). After preparation of the supernatant an aliquot was chromatographed on a silica gel t.l.c. plate and radiolabelled histamine and metabolites were quantified according to the procedure described by Knight & Smith (1978). The major portion of the radioactivity (>97%) chromatographed with an R_F value identical to that of histamine, only a negligible quantity (<3%) chromatographed with N^{τ}-methylhistamine, N^{τ}-methylimidazole-acetic acid or imidazoleacetic acid.

Inotropic studies Atrial biopsy samples obtained from 6 patients during cardiac surgery were prepared for organ bath studies as described by Gristwood *et al.* (1980a). Right atrial trabeculae (dimensions $0.97 \pm 0.12 \times 0.10 \pm 0.01$ cm, $n = 6$) were placed under 1 g resting tension and electrically stimulated to contract at 1 Hz (2 × threshold voltage, 2 ms pulse width) whilst isometric tension was recorded. For each preparation cumulative concentration-response

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curves to histamine 10^{-7} M to 10^{-4} M were obtained to indicate the tissue sensitivity to histamine. After completion of response curves, tissues were washed by overflow and incubated in either normal perfusion medium ($n=3$) or medium containing cimetidine 3.16×10^{-5} M ($n=3$). After approximately 30 min incubation the bath volume was reduced from 10 ml to 4 ml and the total tissue content of the organ bath increased to 200 mg by the addition of a suitable number of pieces of human right atrium approximately 0.3 cm in length and 0.1 cm in diameter. The pieces were threaded onto a length of cotton and positioned in the bath in close proximity to the original electrically driven preparation. These steps were carried out in order to increase both the amount of endogenous histamine for potential release and the ultimate concentration of histamine in the perfusion medium resulting from release. The additional tissue was added to the organ bath after the preliminary histamine exposure in order to minimize possible tissue uptake of exogenous histamine. Five minutes later each preparation was then challenged with compound 48/80, 300 μg/ml, and the inotropic response measured.

Drugs used were cimetidine (SK&F), compound 48/80 (Sigma), and histamine acid phosphate (BDH).

Results *Histamine content of human right atrium* The histamine content of biopsy samples from 3 patients were 1040, 920 and 1146 ng/g fresh wt; mean 1035 ± 65 ng/g fresh wt.

Inotropic studies Each of the six right atrial samples used responded to histamine with concentration-dependent increases in the force of contraction (Figure 1). The samples were divided into two groups, one subsequently incubated with cimetidine, 3.16×10^{-5} M, and one incubated without cimetidine. The mean EC_{50} values were 4.3×10^{-6} M (range $2.6-7.5 \times 10^{-6}$ M) for the control group and 4.7×10^{-6} M ($3.7-7.6 \times 10^{-6}$ M) for the group to be subsequently treated with cimetidine. The maximum responses to histamine 10^{-4} M were 0.53 ± 0.19 g and 0.43 ± 0.01 g respectively.

Administration of compound 48/80 (300 μg/ml) to control preparations caused an immediate increase in the force of contraction, with a peak response occurring approximately 1 min after addition to the bath (Figure 1). The force of contraction subsequently decreased with time. Comparison of the peak response (F max) in each of the untreated group with the corresponding histamine concentration-response curve indicated that the mean response to compound 48/80 was equivalent to the response caused by

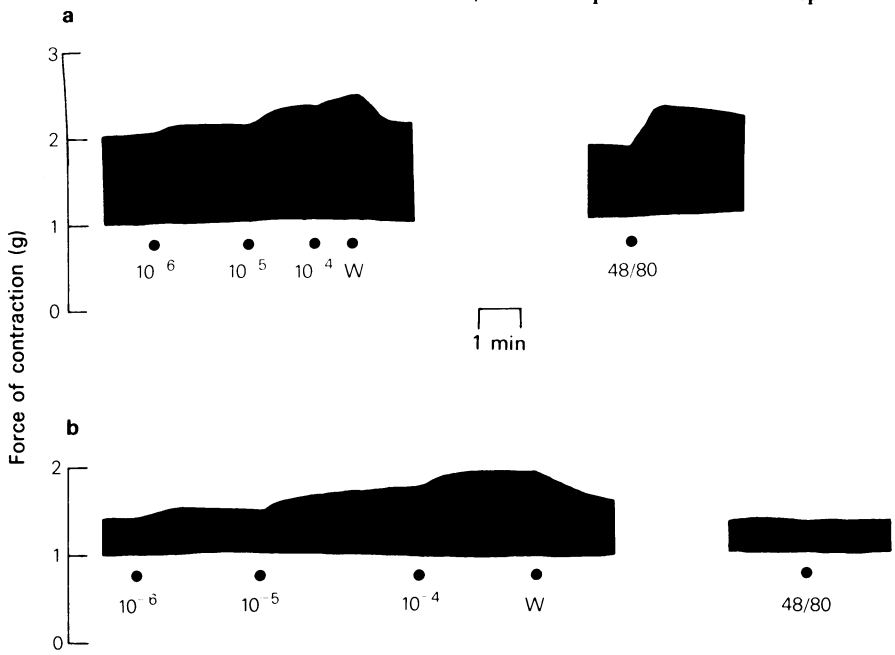

Figure 1 Human right atrium *in vitro*. (a) On the left, histamine 10^{-6}, 10^{-5} and 10^{-4} M, caused concentration-dependent increases in the force of atrial contraction. The tissue was washed at W. On the right in the same preparation, addition of compound 48/80 (300 μg/ml) to the organ bath caused a large increase in the force of atrial contraction. (b) On the left, histamine caused concentration-dependent increases in the force of atrial contraction. Subsequent addition of compound 48/80 (300 μg/ml) to the organ bath, 30 min after incubation with cimetidine, 3.16×10^{-5} M, caused no measurable response.

histamine 4.1×10^{-6} M (range $1.5-7.5 \times 10^{-6}$ M). In contrast, in cimetidine-treated tissues there was no response to compound 48/80 (Figure 1).

Discussion The present experiments demonstrate that human right atrium contains histamine in similar concentrations to that of noradrenaline which is also present in the range 500–2000 ng/g wet weight (Petch & Nayler, 1979), but somewhat less than the histamine content of guinea-pig hearts (Giotti *et al.*, 1966; Gristwood *et al.*, 1980b).

The inotropic response to exogenous histamine in the human right atrium is competitively antagonized by cimetidine and is therefore due to stimulation of histamine H_2-receptors (Gristwood *et al.*, 1980a). The inotropic responses caused by compound 48/80 were similar in time of onset to those for histamine; however, whereas histamine responses were maintained those to compound 48/80 were transient. The transient nature could have been due to the achievement of a localized high concentration of mediator upon release with subsequent diffusion away from the tissue. The inotropic response caused by compound 48/80 in the present study was abolished in tissues treated with cimetidine. A response of similar magnitude to exogenous histamine would have been similarly abolished and the effects of compound 48/80 are therefore attributed to the release of histamine from mast cells within the tissue. It is assumed that all tissue in the bath (electrically driven and quiescent) contributed to the released histamine which produced a response in the electrically driven preparation. We did not investigate whether the original electrically driven preparation alone (ca. 30 mg) contained sufficient releasable histamine to elicit a response. We attempted to support the pharmacological evidence for release of cardiac histamine by assay for histamine in the organ bath contents. Unfortunately, compound 48/80 also present in the contents markedly inhibited the histamine methyltransferase enzyme used in the enzymatic double isotope assay and consequently invalidated the assay.

Previous studies in the guinea-pig have demonstrated that mast cell degranulation can cause major changes in cardiac function including increases in the rate and force of cardiac contraction, decreases in coronary flow and also the production of arrhythmias comprising atrio-ventricular block and ventricular extrasystoles (Levi, 1972; Capurro & Levi, 1973; Gristwood *et al.*, 1980b; Flynn & Owen, 1980) both *in vitro* and *in vivo*. Each of the above changes except the decreases in coronary flow could be attributed to release of mast cell histamine as the responses can be abolished by histamine antagonists. The present studies indicate that the histamine content of the human heart is also potentially labile and can be released in quantities adequate to elicit responses in the tissues from which it has been released. Although release by compound 48/80 represents an experimental procedure, the results indicate that release of endogenous histamine from the human heart can occur. It will be of interest to establish whether other procedures including injury, which release histamine in other tissues, also release sufficient endogenous histamine to modify cardiac function.

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